Figure 1   **Sequence alignment of mouse Serca 1, 2 and 3 protein.**

```
 5   Serca1a    1 MEAAHSKSTEECLSYFGVSETTGLTPDQVKRHLEKYGPNELPAEEGKSLWELVVEQFEDL
     Serca2a    1 ..N..T.TV..V.GH...N.'S...SLE...KLK.RW.S.........T.L...I......
     Serca2b    1 ..N..T.TV..V.GH...N.S...SLE...KLK.RW.S.........T.L...I......
     Serca3a    1 ..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.......T.................
     Serca3b    1 ..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.......T.................
10   Serca3c    1 ..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.......T.................

     Serca1a   61 LVRILLLAACISFVLAWFEEGEETVTAFVEPFVILLILIANAIVGVWQERNAENAIEALK
     Serca2a   61 .......................I...............V....................
     Serca2b   61 .......................I...............V....................
15   Serca3a   61 .........LV.............T......L..M...V..............S......
     Serca3b   61 .........LV.............T......L..M...V..............S......
     Serca3c   61 .........LV.............T......L..M...V..............S......

     Serca1a  121 EYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSIL
20   Serca2a  121 ...........Q...........K..........I...........LT............
     Serca2b  121 ...........Q...........K..........I...........LT............
     Serca3a  121 .........I.S...G....R...................L.LIE..............
     Serca3b  121 .........I.S...G....R...................L.LIE..............
     Serca3c  121 .........I.S...G....R...................L.LIE..............
25
     Serca1a  181 TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAVGIVATTGVSTEIGKIRDQMA
     Serca2a  181 ..........................................M.V.VA...N........E.V
     Serca2b  181 ..........................................M.V.VA...N........E.V
     Serca3a  181 .......T....AI.....................S...L.VAVA..LQ..L....S...
30   Serca3b  181 .......T....AI.....................S...L.VAVA..LQ..L....S...
     Serca3c  181 .......T....AI.....................S...L.VAVA..LQ..L....S...

     Serca1a  241 ATEQDKTPLQQKLDEFGEQLSKVISLICVAVWLINIGHFNDPVHGGSWFRGAIYYFKIAV
     Serca2a  241 ....ER.....................I...I...............I............
35   Serca2b  241 ....ER.....................I...I...............I............
     Serca3a  241 .V.PER....R......R...HA..V......V......A..A.....L...V.......
     Serca3b  241 .V.PER....R......R...HA..V......V......A..A.....L...V.......
     Serca3c  241 .V.PER....R......R...HA..V......V......A..A.....L...V.......

40   Serca1a  301 ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ
     Serca2a  301 ............................................................
     Serca2b  301 ............................................................
     Serca3a  301 ..........................R.................................
     Serca3b  301 ..........................R.................................
45   Serca3c  301 ..........................R.................................

     Serca1a  361 MSVCKMFIIDKVDGDVCSLNEFSITGSTYAPEGEVLKNDKPVRAGQYDGLVELATICALC
     Serca2a  361 ....R...L...E..T................I...Q.D....KCH..............
     Serca2b  361 ....R...L...E..T................I...Q.D....KCH..............
50   Serca3a  361 ....R..VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F..............
     Serca3b  361 ....R..VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F..............
     Serca3c  361 ....R..VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F..............

     Serca1a  421 NDSSLDFNETKGVYEKVGEATETALTTLVEKMNVFNTEVRSLSKVERANACNSVIRQLMK
55   Serca2a  421 ...A..Y..A...............C...........D..LKG...I.........K....
     Serca2b  421 ...A..Y..A...............C...........D..LKG...I.........K....
     Serca3a  421 ...A..Y..A...............C.......D.DLKG..R....G......K...R
     Serca3b  421 ...A..Y..A...............C.......D.DLKG..R....G......K...R
     Serca3c  421 ...A..Y..A...............C.......D.DLKG..R....G......K...R
```

```
     Serca1a  481 KEFTLEFSRDRKSMSVYCSPAKSSRAAVGNKMFVKGAPEGVIDRCNYVRVGTTRVPLTGP
     Serca2a  481 ................T.N.P..TSMS-...............THI...S.K..M.PG
     Serca2b  481 ................T.N.P..TSMS-...............THI...S.K..M.PG
     Serca3a  481 ................T.TRADPKVQ.S.........S..E..SS....SRTA..STT
  5  Serca3b  481 ................T.TRADPKVQ.S.........S..E...SS....SRTA..STT
     Serca3c  481 ................T.TRADPKVQ.S.........S..E..SS....SRTA..STT


     Serca1a  541 VKEKIMSVIKEWGTGRDTLRCLALATRDTPPKREEMVLDDSAKFMEYEMDLTFVGVVGML
     Serca2a  540 ..Q......R...S.S...........H.N.L.....H.E...N.IK..TN.....C....
 10  Serca2b  540 ..Q......R...S.S...........H.N.L.....H.E...N.IK..TN.....C....
     Serca3a  541 SR.H.LAK.RD..S.S.............RK.D.H...CSR.VQ..T......C....
     Serca3b  541 SR.H.LAK.RD..S.S.............RK.D.H...CSR.VQ..T......C....
     Serca3c  541 SR.H.LAK.RD..S.S.............RK.D.H...CSR.VQ..T......C....


 15  Serca1a  601 DPPRKEVTGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGIFSENEEVTDRAYTGREFDD
     Serca2a  600 ....I..AS.VK...Q.................V.........GQD.D..SK.F.....E
     Serca2b  600 ....I..AS.VK...Q.................V.........GQD.D..SK.F.....E
     Serca3a  601 ....P..AAC.TR.SR.....V..........V.....L...GDT.D.LGK.........
     Serca3b  601 ....P..AAC.TR.SR.....V..........V.....L...GDT.D.LGK.........
 20  Serca3c  601 ....P..AAC.TR.SR.....V..........V.....L...GDT.D.LGK.........


     Serca1a  661 LPLAEQREACRRACCFARVEPSHKSKIVEYLQSYDEITAMTGDGVNDAPALKKAEIGIAM
     Serca2a  660 .SPSA..D..LN.R..............F...F..................S......
     Serca2b  660 .SPSA..D..LN.R..............F...F..................S......
 25  Serca3a  661 .SPEQ..Q...T.R.......A...R...N...FN....................
     Serca3b  661 .SPEQ..Q...T.R.......A...R...N...FN....................
     Serca3c  661 .SPEQ..Q...T.R.......A...R...N...FN....................


     Serca1a  721 GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA
 30  Serca2a  720 ............................................................
     Serca2b  720 ............................................................
     Serca3a  721 ........S.A....S....AS.............................I
     Serca3b  721 ........S.A....S....AS.............................I
     Serca3c  721 ........S.A....S....AS.............................I
 35
     Serca1a  781 LGLPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMDRPPRSPKEPLISGWLFFRYMAI
     Serca2a  780 ..F..........................NK...N.............L..
     Serca2b  780 ..F..........................NK...N.............L..
     Serca3a  781 .............................EK...N.R.A.........L..
 40  Serca3b  781 .............................EK...N.R.A.........L..
     Serca3c  781 .............................EK...N.R.A.........L..


     Serca1a  841 GGYVGAATVGAAAWWFLYAEDGPHVSYHQLTHFMQCTEHNPEFDGLDCEVFEAPEPMTMA
     Serca2a  840 .C..............IA.DG..R..FY..S..L..K.D..D...V..AI..S.Y.....
 45  Serca2b  840 .C..............IA.DG..R..FY..S..L..K.D..D...V..AI..S.Y.....
     Serca3a  841 .V...L...A..T.....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...
     Serca3b  841 .V...L...A..T.....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...
     Serca3c  841 .V...L...A..T.....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...


 50  Serca1a  901 LSVLVTIEMCNALNSLSENQSLLRMPPWVNIWLLGSICLSMSLHFLILYVDPLPMIFKLR
     Serca2a  900 ..........................E....V.................E...L..QIT
     Serca2b  900 ..........................E....V.................E...L..QIT
     Serca3a  901 ...............V..........L.P....AVVM..A......L.P...L..QVT
     Serca3b  901 ...............V..........L.P....AVVM..A......L.P...L..QVT
 55  Serca3c  901 ...............V..........L.P....AVVM..A......L.P...L..QVT
```

```
     Serca1a   961 ALDFTQWLMVLKISLPVIGLDELLKFIARNYLEG
     Serca2a   960 P.NL...............LM..T...V......QPAILE
     Serca2b   960 P.NL...............LM..T...V......QPGKECVQPATKSSCSLSACTDGISWP
     Serca3a   961 P.SGR..GV..QM.....L...A..YLS..HMDEKKDLK
  5  Serca3b   961 P.SGR..GV..QM.....L...A..YLS..HMD.VLGTFMQARSRQLPTTSRTPYHTGKK
     Serca3c   961 P.SGR..GV..QM.....L...A..YLS..HMD.VLGTFMQARSRQLPTTSRTPYHTGLA


     Serca2b  1020 FVLLIMPLVVWVYSTDTNFSDMFWS
 10  Serca3b  1021 GPEVNPGSRGESPVWPSD
     Serca3c  1021 SWKKRT
```

15

Figure 2   Sequence similarity of Serca2 proteins in mammalian species

```
        Mouse_2a     1 MENAHTKTVEEVLGHFGVNESTGLSLEQVKKLKERWGSNELPAEEGKTLLELVIEQFEDL
 5      Mouse_2b     1 ...........................................................
        Rat_2b       1 ...........................................................
        Rat_2a       1 ...........................................................
        Dog_2a       1 ...........................................................
        Cat_2a       1 ...........Y...............................................
10      Pig_2a       1 ...........................................................
        Pig_2b       1 ...........................................................
        Human_2b     1 ...........................................................
        Human_2c     1 ...........................................................
        Human_2a     1 ...........................................................
15      Rabbit_2a    1 ...........................................................
        Rabbit_2b    1 ...........................................................


        Mouse_2a    61 LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILVANAIVGVWQERNAENAIEALK
        Mouse_2b    61 ...........................................................
20      Rat_2b      61 ...........................................................
        Rat_2a      61 ...........................................................
        Dog_2a      61 ...........................................................
        Cat_2a      61 ...........................................................
        Pig_2a      61 ...........................................................
25      Pig_2b      61 ...........................................................
        Human_2b    61 ...........................................................
        Human_2c    61 ...........................................................
        Human_2a    61 ...........................................................
        Rabbit_2a   61 ...........................................................
30      Rabbit_2b   61 ...........................................................


        Mouse_2a   121 EYEPEMGKVYRQDRKSVQRIKAKDIVPGDIVEIAVGDKVPADIRLTSIKSTTLRVDQSIL
        Mouse_2b   121 ...........................................................
        Rat_2b     121 ...........................................................
35      Rat_2a     121 ...........................................................
        Dog_2a     121 ...........................................................
        Cat_2a     121 ...........................................................
        Pig_2a     121 ...........................................................
        Pig_2b     121 ...........................................................
40      Human_2b   121 ...........................................................
        Human_2c   121 ...........................................................
        Human_2a   121 ...........................................................
        Rabbit_2a  121 ...........................................................
        Rabbit_2b  121 ...........................................................
45
        Mouse_2a   181 TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAMGVVVATGVNTEIGKIRDEMV
        Mouse_2b   181 ...........................................................
        Rat_2b     181 ...........................................................
        Rat_2a     181 ...........................................................
50      Dog_2a     181 ...........................................................
        Cat_2a     181 ...........................................................
        Pig_2a     181 ...........................................................
        Pig_2b     181 ...........................................................
        Human_2b   181 ...........................................................
55      Human_2c   181 ...........................................................
        Human_2a   181 ...........................................................
        Rabbit_2a  181 ...........................................................
        Rabbit_2b  181 ...........................................................
```

```
     Mouse_2a    241 ATEQERTPLQQKLDEFGEQLSKVISLICIAVWIINIGHFNDPVHGGSWIRGAIYYFKIAV
     Mouse_2b    241 ............................................................
     Rat_2b      241 ............................................................
 5   Rat_2a      241 ............................................................
     Dog_2a      241 ............................................................
     Cat_2a      241 ............................................................
     Pig_2a      241 ............................................................
     Pig_2b      241 ............................................................
10   Human_2b    241 ............................................................
     Human_2c    241 ............................................................
     Human_2a    241 ............................................................
     Rabbit_2a   241 ............................................................
     Rabbit_2b   241 ............................................................

15
     Mouse_2a    301 ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ
     Mouse_2b    301 ............................................................
     Rat_2b      301 ............................................................
     Rat_2a      301 ............................................................
20   Dog_2a      301 ............................................................
     Cat_2a      301 ............................................................
     Pig_2a      301 ............................................................
     Pig_2b      301 ............................................................
     Human_2b    301 ............................................................
25   Human_2c    301 ............................................................
     Human_2a    301 ............................................................
     Rabbit_2a   301 ............................................................
     Rabbit_2b   301 ............................................................


30   Mouse_2a    361 MSVCRMFILDKVEGDTCSLNEFSITGSTYAPIGEVQKDDKPVKCHQYDGLVELATICALC
     Mouse_2b    361 ............................................................
     Rat_2b      361 .........................T..................................
     Rat_2a      361 .........................T..................................
     Dog_2a      361 ..........R....S.........T..............H...................
35   Cat_2a      361 .........................T..............H...................
     Pig_2a      361 .........................T..............H...................
     Pig_2b      361 .........................T..............H...................
     Human_2b    361 ..........R..............T..............H.......N...........
     Human_2c    361 ..........R..............T..............H.......N...........
40   Human_2a    361 ..........R..............T..............H.......N...........
     Rabbit_2a   361 ...........D.............T..............H...................
     Rabbit_2b   361 ...........D.............T..............H...................


     Mouse_2a    421 NDSALDYNEAKGVYEKVGEATETALTCLVEKMNVFDTELKGLSKIERANACNSVIKQLMK
45   Mouse_2b    421 ............................................................
     Rat_2b      421 ............................................................
     Rat_2a      421 ............................................................
     Dog_2a      421 ............................................................
     Cat_2a      421 ...............K.F..........................................
50   Pig_2a      421 ............................................................
     Pig_2b      421 ............................................................
     Human_2b    421 ............................................................
     Human_2c    421 ............................................................
     Human_2a    421 ............................................................
55   Rabbit_2a   421 ............................................................
     Rabbit_2b   421 ............................................................
```

```
        Mouse_2a   481  KEFTLEFSRDRKSMSVYCTPNKPSRTSMSKMFVKGAPEGVIDRCTHIRVGSTKVPMTPGV
        Mouse_2b   481  ...........................................................
        Rat_2b     481  ...........................................................
        Rat_2a     481  ...........................................................
   5    Dog_2a     481  ...........................................................
        Cat_2a     481  ...........................................................
        Pig_2a     481  ...........................................................
        Pig_2b     481  ...........................................................
        Human_2b   481  ........................................................S..
   10   Human_2c   481  ........................................................S..
        Human_2a   481  ........................................................S..
        Rabbit_2a  481  ........................................................A..
        Rabbit_2b  481  ........................................................A..


   15   Mouse_2a   541  KQKIMSVIREWGSGSDTLRCLALATHDNPLKREEMHLEDSANFIKYETNLTFVGCVGMLD
        Mouse_2b   541  ...........................................................
        Rat_2b     541  .............................R.............................
        Rat_2a     541  .............................R.............................
        Dog_2a     541  ...V.........................R....N........................
   20   Cat_2a     541  ...V.........................R....N........................
        Pig_2a     541  ............................MR....N........................
        Pig_2b     541  ............................MR....N........................
        Human_2b   541  .............................R.............................
        Human_2c   541  .............................R.............................
   25   Human_2a   541  .............................R.............................
        Rabbit_2a  541  .............................R.....K.......................
        Rabbit_2b  541  .............................R.....K.......................


        Mouse_2a   601  PPRIEVASSVKLCRQAGIRVIMITGDNKGTAVAICRRIGIFGQDEDVTSKAFTGREFDEL
   30   Mouse_2b   601  ...........................................................
        Rat_2b     601  ...........................................................
        Rat_2a     601  ...........................................................
        Dog_2a     601  ...........................................................
        Cat_2a     601  ...........................................................
   35   Pig_2a     601  ...........................................................
        Pig_2b     601  ...........................................................
        Human_2b   601  ...........................................................
        Human_2c   601  ...........................................................
        Human_2a   601  ...........................................................
   40   Rabbit_2a  601  ...................................................E....A..
        Rabbit_2b  601  ...................................................E....A..


        Mouse_2a   661  SPSAQRDACLNARCFARVEPSHKSKIVEFLQSFDEITAMTGDGVNDAPALKKSEIGIAMG
        Mouse_2b   661  ...........................................................
   45   Rat_2b     661  ...........................................................
        Rat_2a     661  ...........................................................
        Dog_2a     661  ...........................................................
        Cat_2a     661  ...........................................................
        Pig_2a     661  N.....E....................................................
   50   Pig_2b     661  N.....E....................................................
        Human_2b   661  N.............................................A............
        Human_2c   661  N.............................................A............
        Human_2a   661  N.............................................A............
        Rabbit_2a  661  N.............................................A............
   55   Rabbit_2b  661  N.............................................A........
```

```
    Mouse_2a   721  SGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAAL
    Mouse_2b   721  ...........................................................
    Rat_2b     721  ...........................................................
    Rat_2a     721  ...........................................................
 5  Dog_2a     721  ...........................................................
    Cat_2a     721  ...........................................................
    Pig_2a     721  ...........................................................
    Pig_2b     721  ...........................................................
    Human_2b   721  ...........................................................
10  Human_2c   721  ...........................................................
    Human_2a   721  ...........................................................
    Rabbit_2a  721  ...........................................................
    Rabbit_2b  721  ...........................................................

15  Mouse_2a   781  GFPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMNKPPRNPKEPLISGWLFFRYLAIG
    Mouse_2b   781  ...........................................................
    Rat_2b     781  ...........................................................
    Rat_2a     781  ...........................................................
    Dog_2a     781  ...........................................................
20  Cat_2a     781  ...........................................................
    Pig_2a     781  ...........................................................
    Pig_2b     781  ...........................................................
    Human_2b   781  ...........................................................
    Human_2c   781  ...........................................................
25  Human_2a   781  ...........................................................
    Rabbit_2a  781  ...........................................................
    Rabbit_2b  781  ...........................................................

    Mouse_2a   841  CYVGAATVGAAAWWFIAADGGPRVSFYQLSHFLQCKEDNPDFDGVDCAIFESPYPMTMAL
30  Mouse_2b   841  ...........................................................
    Rat_2b     841  ....................................................E......
    Rat_2a     841  ....................................................E......
    Dog_2a     841  ..............................................D.....E......
    Cat_2a     841  ..............................................D.....E......
35  Pig_2a     841  ...................................T................E.....V.........
    Pig_2b     841  ...................................T................E.....V.........
    Human_2b   841  ....................................................E......
    Human_2c   841  ....................................................E......
    Human_2a   841  ....................................................E......
40  Rabbit_2a  841  ....................................................E......
    Rabbit_2b  841  ....................................................E......

    Mouse_2a   901  SVLVTIEMCNALNSLSENQSLLRMPPWENIWLVGSICLSMSLHFLILYVEPLPLIFQITP
    Mouse_2b   901  ...........................................................
45  Rat_2b     901  ...........................................................
    Rat_2a     901  ...........................................................
    Dog_2a     901  ...........................................................
    Cat_2a     901  ...........................................................
    Pig_2a     901  ...........................................................
50  Pig_2b     901  ...........................................................
    Human_2b   901  ...........................................................
    Human_2c   901  ...........................................................
    Human_2a   901  ...........................................................
    Rabbit_2a  901  ...........................................................
55  Rabbit_2b  901  ...........................................................
```

```
     Mouse_2a    961 LNLTQWLMVLKISLPVILMDETLKFVARNYLEQPAILE----------------------
     Mouse_2b    961 ..................................GKECVQPATKSSCSLSACTDGISWPF
     Rat_2b      961 ...............................-.GKECA.....P..............
     Rat_2a      961 ...............................-.AILE
  5  Dog_2a      961 ...............................-.AILE
     Cat_2a      961 ...............................-.AILE
     Pig_2a      961 ...............................-.AILE
     Pig_2b      961 ...............................-.GKEC.......-..F..........
     Human_2b    961 ..V............................-.GKEC.......-..F..........
 10  Human_2c    961 ..V............................-.VLSSL
     Human_2a    961 ..V............................-.AILE
     Rabbit_2a   961 ..V............................-.AILE
     Rabbit_2b   961 ..V............................-.GKEC....PQ.-...W...E.V....


 15  Mouse_2b   1021 VLLIMPLVVWVYSTDTNFSDMFWS
     Rat_2b     1020 ........................
     Pig_2b     1019 ........................
     Human_2b   1019 ........................
     Rabbit_2b  1019 ....V...M...........LL..
 20
```

Fig. 3 Targeting construct for Serca2 flox gene modification. Sequence information.

------------------------ Serca2 gene ------------------------------------

LoxP site 1: Intron 1

underlined sequence = loxP site and cloning sequence

Exon I (partial)

CACGGGGCTGAGCTTGGAGCAGGTCAAGAAGCTCAAGGAGAGATGGGGCTCCAACG*gtaggtgcggggcg*
ccggggctgcagcggcgcggcgcgggcccgagcgccaaggaagatggctgacccggctccacctcgtggg
gcttggctcggcgcggcgcccgacggctgcgagaggccggcggtccacgcgcgggtctgggccatcgccg
*accttaggggt*ctcgaatcaagcttatcgataccgtcgatcggacctcgaggggggggcccggtacccggg
gatcaattcgagctcgcccggggatcgatccggaacccttaat**ATAACTTCGTATAATGTATGCTATACG**
AAGTTATtaggtccctcgacctgcagcccaagctccGGGGAtctcgagccggtgaccttccggccggcg
ctcagcgagtcggattgggggggggggagagggagtgggaaggaggggaggaggttcctgcggctgggctg
agtcccccgcggatttatgaggcgtcgatgttgttgagaaaccctcggaccgtttcttgtgctccccaaa
gttgcacatctggcagaagtgatgacccagctgaaatgactgcatggtcctggaggccggagagggctta
cgggcagttccgaggccactgattaccagggctgaataattttctcggggtatcaaagtggagacagatt
gttgtacgttcatacacctatatccgccattcagacaacgatggtggtgaatttagcagttttaataaa
agcgctaatacaatatcttcattttctttc

------------------------ Serca2 gene ------------------------------------

LoxP site 2: Intron 3 5′ of genomic XcmI site

underlined sequence = loxPsite, cloning sites and partial HSV-TK

ccaatttttattcttagaacattgtattcttatactgtgataggaagtgaaaaatcatacagtacttgtc
ttaggtttcacaaaactgataactgtatggtttcaattatgtattcacacgtttaagtctgacccagggG
GATCCggaacccttaat**ATAACTTCGTATAATGTATGCTATACGAAGTTAT**taggtccctcgacctgcag
cccaagctgatcctctagtcgagccccagctggttctttccgcctcagaagccatagagccaccgcatc
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gatgcgctgcgaatcgggagcggcgat*ccgtaaagcacgaggaagcgg*cagcccattcgccgccaagc
tctttcagcaatatcacgggtagccaacgctatgt*ctgataagcggtccgcccacacccaa*ccggcca
caagtc*atgaaatcca*aaaaagcgggccattttttccacc*atgatttt*cggcaagcaaggccttt*c
cattgggtcaccgac*aga*catt*tccgt*c*ggcattgcgc*ccct

------------------------ HSV-TK Neo antibiotics cassette ------------------------

LoxP site 3: Intron 3 5′ of genomic XcmI site

underlined sequence = loxP site, cloning sites and partial Neo gene

gtttttcat*accaccgcgggtcccgggc*gatat*ttcaccttgtc*ag*cggtgttgtgtggtgtaaatg
ttcgcgattgtttcgaaagccc*agcacccgccagtaagtcatcggctcgggtacgtagacgatatcgtc
gcgcgaacccagggccaccagcaagttgcgtggtggtggttttccccatcc*gtggggac*gtctatataa
acc*gcagtagcgtgggcatttttctgctccgggcggacttccgtggcttcttgctgccggcgagggcgcaa
cgccgtacgtcggttgctatggccgcgagaacgcgcagcctggtcgaacgcagacgcgtgttgatggccgg
ggtacgaagccatacgcgcttctacaaggcgctggccgaagaggtgcgggagtttcacgccaccaagatct
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ttaatatgcgaagtggacctcggaccgcgccgcccgactgcatctgcgtgttcgaattcgccaatgacaa
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tcttgcaaaaccacactgctcgacattgggtggaaacattccaggcctgggtggagaggcttttttgcttcc
tcttgaaaaccacactgctcgatccggaacccttaat**ATAACTTCGTATAATGTATGCTATACGAA**
GTTATtaggtccctcgacctgcagcccaagctgatcctctagagtcgacctcga*tctgtggtcatggcctctatgaaa*
acattagcttagagg

Fig. 4A   **Schematic representation of genetic manipulation.**

Serca2 (*atp2a2*) gene modification


5
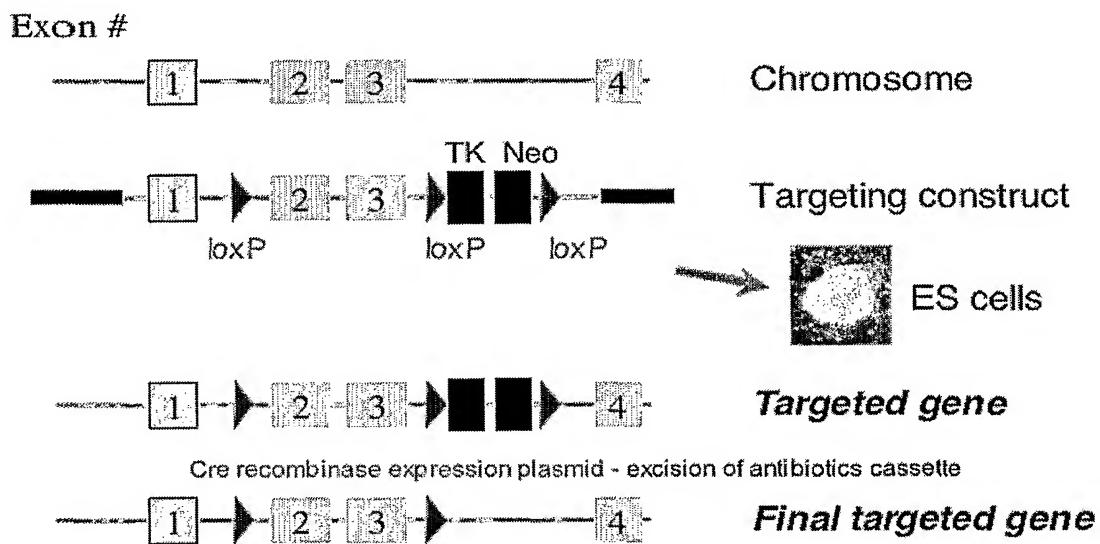
Fig 4 B: Verification of Serca locus targeting events offspring from chimeric mice.

5
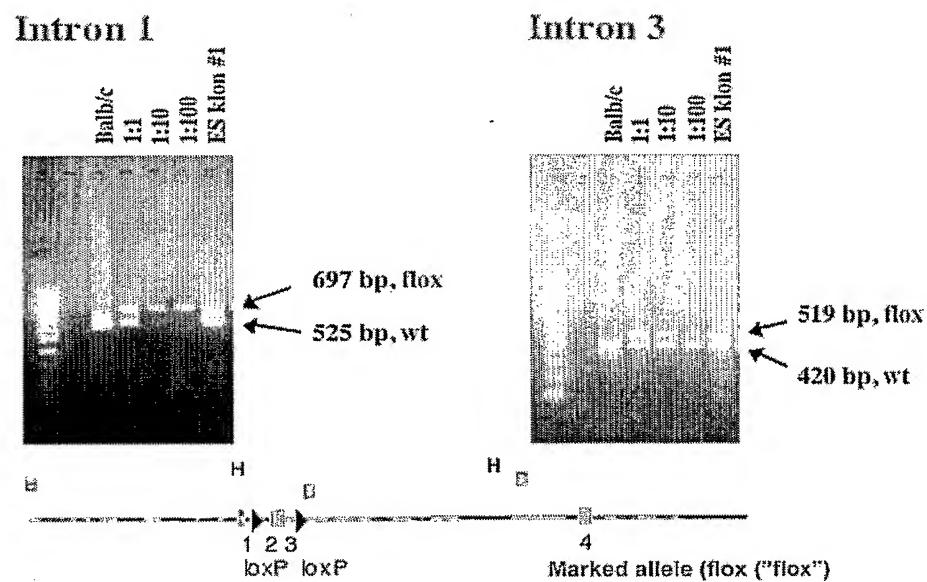
Fig. 5 Specificity of gene deletion in a test model.

5
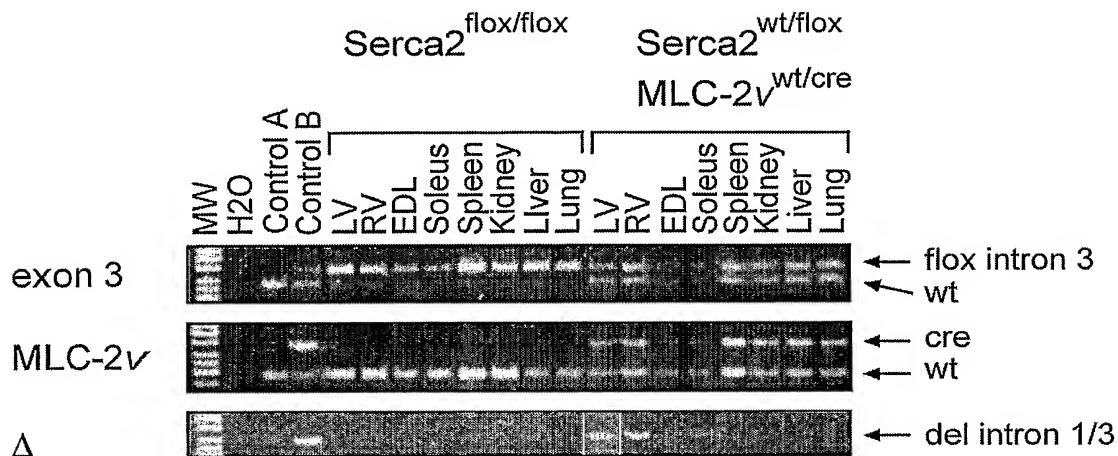
Control A =   wt ES cells (14.1a)

Control B =   Upper panel: Serca2$^{wt/flox}$ ES cells

Middle panel: Left ventricle from MLC-2V-Cre mice

Lower panel: Serca2$^{wt/del}$ ES cells

LV = heart left ventricle

RV = heart right ventricle

EDL = extensor digitorum longus muscle (fast-twitch skeletal muscle)

Soleus = soleus muscle (slow-twitch skeletal muscle)

Other tissues as indicated.

Fig. 6 Cardiac ANP mRNA expression.

5

Fig. 7 Serca2 protein expression.
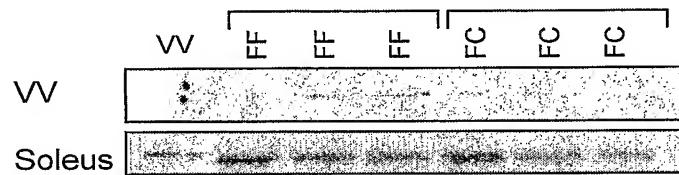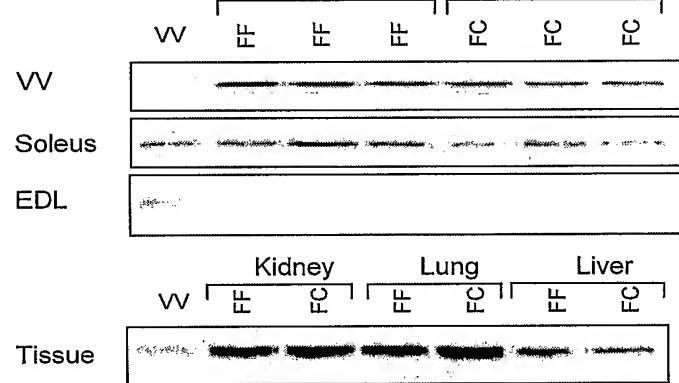
Fig. 8 Compensatory mechanisms in Serca^{flox} MLC-*2v*-Cre mice.

A

PLB total

P–Ser16

P–Thr17

B

pentamer

monomer

PLB total

pentamer

monomer

P-Ser16

pentamer

monomer

P-Thr17

C

VV

Soleus

EDL
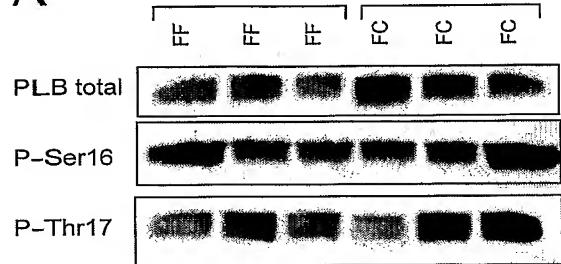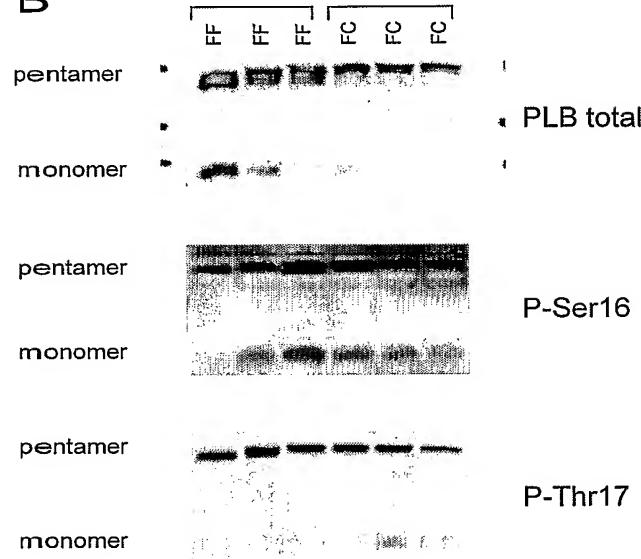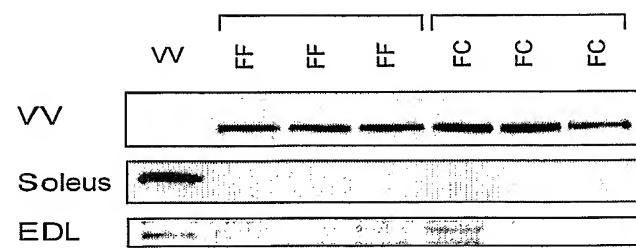
Figure 9   Genotypes PCR
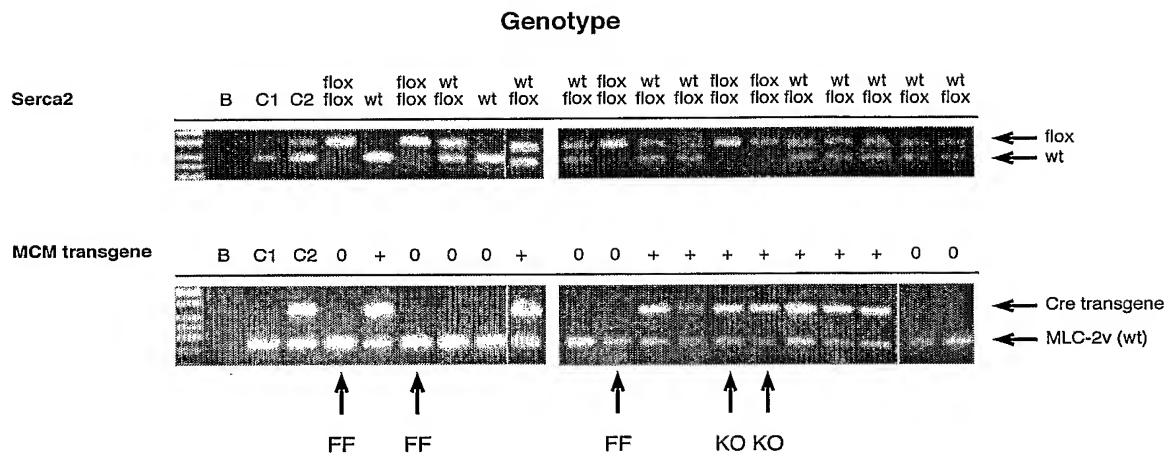
Generation of animals with Serca2^{flox} and MCM transgene alleles.

Genotypes FF. Serca2$^{flox/flox}$;   KO, Serca2$^{flox/flox}$ MCM

Figure 10   **Heart morphology**

5
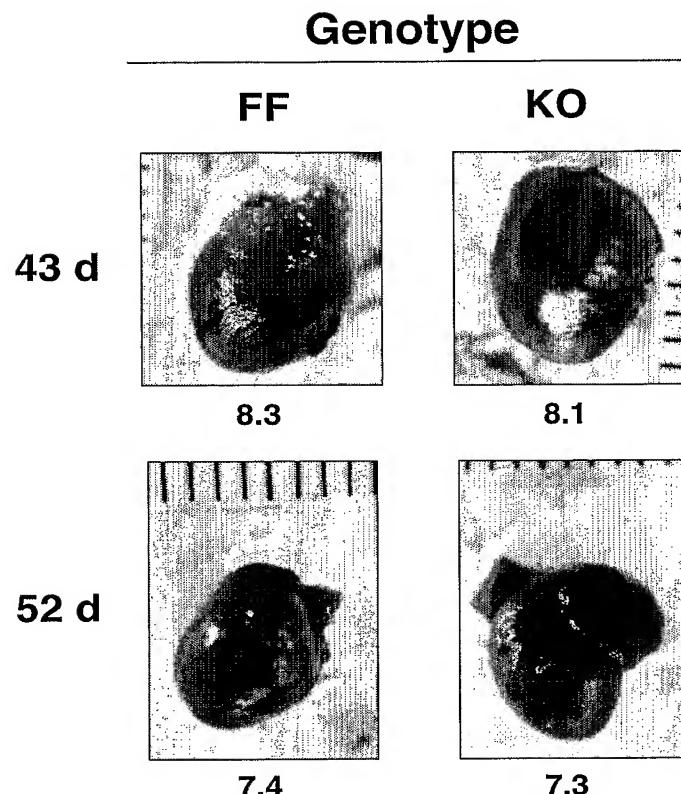
10

Figure 11   Pilot series left atrial diastolic diameter.

5

Tamoxifen-injected animals
43-52 days
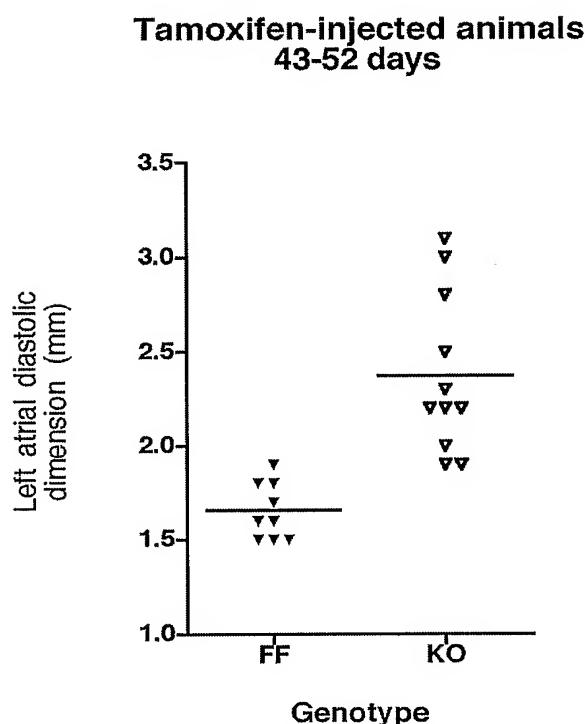
10

15

Figure 12   **Serca protein content in tamoxifen-induced FF and KO mice**

A

B